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Preliminary Note on Leaf Invasions by Bacillus amylovorus

By

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Preliminary Note on Leaf Invasions by Bacillus amylovorus

BY

FREDERICK D. HEALD, PATHOLOGIST.

HISTORICAL STATEMENT.

Investigators who have made a special study of fire blight or pear blight due to *Bacillus amylovorus* agree that the disease manifests itself in the four following ways according to the parts invaded:

1. **Blossom blight**, due to original infection through the nectaries by bacteria disseminated by insects which visit the flowers in search of food.

2. **Twig blight**, due to infection through wounds made by insects or other agents in the succulent tissue of terminal shoots.

3. **Fruit blight**, due to primary infections through some wound or migration of the bacteria up the pedicel into the pulp of the fruit.

4. **Cankers** or **body blight**, due to migration of the bacillus down fruit spurs, from twigs or water sprouts showing **twig blight** or by primary infections through wounds.

The only intimation that the writer has found concerning the probability of direct invasion through the leaves is contained in the following statement[1] by Duggar:

"Nevertheless, it is also true that infection may result through growing twigs. Biting or piercing insects are doubtless of much importance in spreading the disease in this way. Injuries and sometimes, perhaps, even water-pores may be the seats of infection. In general, however, it is certainly true that the presence of germs upon the surface of healthy tissues would not result in the production of disease in those parts."

The above statement appears to admit the possibility of invasion through the water-pores, although no observations or experiments are offered in substantiation of this behavior of the blight bacillus.

The general opinion of pathologists in regard to leaf in-

1. Duggar, B. M. Fungous diseases of plants, p. 125, 1909.

vasion is that presented by Waite[1]. He states first that "while the bacteria themselves rarely kill the leaves, at most only occasionally attacking stems and midribs of the youngest ones, all the foliage of the blighted branches must of course eventually die." A proper interpretation of this statement would mean that sometimes the bacteria migrate from the stem up the petiole and into the mid-rib of the leaf, but certainly does not suggest a downward migration from an original leaf infection. Concerning the origin of twig blight in nursery stock or young trees which have not yet blossomed the same author[2] writes, "Another way in which the blight gains entrance is through the tips of growing shoots. In the nursery when the trees are not flowering this is the usual mode of infection."

It is generally conceded that the infections in the vegetative shoots are caused by bacteria introduced by biting or sucking insects. Whetzel and Stewart[3] write. "Our own observations tend to show that the aphides and leaf hoppers are largely the responsible agents in introducing the bacteria into the tips of growing shoots, while the same insects and the curculio frequently introduce them into wounds which they make in the fruit, thus giving rise to Fruit Blight." The same writers[4] continue, "During rainy weather the bacteria ooze from these blighted blossoms and are carried by plant lice, leaf hoppers, and other sucking insects to the tips of the twigs that are now growing rapidly; here in sucking the sap the insect introduces the bacteria into the tender tissues where they multiply rapidly, producing in a few days the characteristic 'Twig Blight.'"

The possibility of either stomatal or water-pore invasion would seem to be excluded by Whetzel[5] as is evidenced by the following quotations: "As a general deduction, then, it may be stated that infection occurs only through a wound of some sort." This opinion is presented in a discusion of the origin of body blight. Whetzel and Stewart[6] state later that, "It is certain that unless these bacteria are introduced into the trees by insects or by the grower himself, no blight will result."

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1. Waite, M. B. The cause and prevention of pear blight. Yearbook U. S. Dept. of Agr., 1895, p. 295-296. 1896
2. Ibid, p. 297.
3. Whetzel, H. H. and Stewart, V. B. Fire blight of pears, apples, quinces, etc. Bul. Cornell Univ. Agr. Exp. Sta., 272: 39-40. 1909.
4. Ibid, p. 41.
5. Whetzel, H. H. The blight canker of apple trees. Bul. Cornell Univ. Agr. Exp. Stat., 236: 119. 1906.
6. Ibid, 43.

Fig. 1. Apple leaves showing characteristic terminal and marginal invasions by *Bacillus amylovorus.*

Fig. 2. Apple leaves showing central lesions caused by *Bacillus amylovorus.*

The recent investigations by Bachmann[1] on the migration of *Bacillus amylovorus* in the host tissue would seem to pave the way for stomatal or water-pore invasion, since this investigator has shown that the bacteria migrate in the intercellular spaces, rather than by penetration of the cells. It may be noted also that this writer has shown that the blight bacillus enters the vascular bundles and migrates along the vessels of the xylem.

A statement made by Arthur in reporting on his early investigations of blight[2] has not been disputed by later workers. "Bacteria can not be found swarming in the leaves as in the bark and wood; the conditions do not seem favorable for their development."

OBSERVATIONS AND EXPERIMENTS RELATING TO LEAF INVASIONS.

During the past season the writer has had an opportunity to visit a number of the apple and pear growing regions of Eastern Washington. As a result of some preliminary studies made in the field and supplemented by laboratory tests it can be stated that leaf invasions by *Bacillus amylovorus* are common.

The first observations were made at North Yakima on July 6th. Leaves of Bartlett pears showing lesions advancing from the edge or tips were collected. During the following night there was a shower and similiar material collected early the next morning showed drops of bacterial exudate. On July 10th and 11th an examination was made of blight infested apple orchards in the vicinity of Spokane. Very similar leaf invasions were found upon Wagener, Jonathan, and Rome Beauty trees. The orchards at North Yakima were irrigated, while those visited at Spokane were not. Later collections have been made at Walla Walla, Kennewick, and Prosser from both pear and apple trees.

In the majority of cases the leaf infections start at the margin and are either lateral or terminal, (Fig. 1) although central lesions have been found in some cases on apple leaves. (Fig. 2). The lesions on the apple leaves are a light brown or yellowish brown and frequently show a faint purplish border at the advancing edge. In acitive lesions the advancing edge shows a narrow watery zone. Those on pear

1. Bachmann, Freda M. The migration of Bacillus amylovorus in the host tissue. Phytopathology 3: 3-13. 1913.
2. Arthur, J. C. Pear blight and its causes. Am. Naturalist, p. 1178. 1885.

leaves are darker in color and exhibit a mottling of various shades of dirty brown. There is a noticeable tendency for the bacteria to advance more rapidly down the midrib or certain lateral veins, so that many young lesions are more or less triangular in outline. In some cases the migration of the bacteria can be noticed along certain veins in advance of the general border of the dead area.

All stages of leaf invasions have been found from slight marginal infections to lesions which have advanced throughout the entire leaf blade and down the petiole. These leaf infections were not rare but it was possible to find dozens of them on a single five-year-old tree. The writer is of the opinion that the bacteria enter the intercellular spaces through the water-pores and also by the stomata to some extent and later penetrate the vessels in the way suggested by Bachmann[1]. It remains for further investigation to definitely substantiate this view.

It is an easy matter to verify the presence of the bacteria by microscopic examination. Dissections made from the advancing edge of a lesion give the organisms in large numbers and if the tissue selected includes one of the larger veins they can be seen to ooze out from the broken ends of the vessels. A sufficient number of lesions have been examined to leave no doubt as to the constant presence of the bacteria.

The lesions have also been tested by cultures for the presence of living bacteria. Mr. H. W. Samson, Horticultural Inspector at Spokane, assisted in collecting material and also sent fresh specimens to our laboratory for use. It was at his solicitation that the writer first visited Spokane to make field observations. In many of the isolations tried the bacteria were found to be dead, but pure cultures were obtained from others by the poured plate method. (Fig. 3). Since the study of these leaf lesions was not begun until July, this condition is what one would expect, as at this time of the year the bacteria are dead in a good per cent of the twig infections. In some cases where microscopic examination showed an abundance of bacteria, the cultures showed that only a relatively small per cent were alive. It seems probable that a certain per cent of the leaf lesions will behave like the twig lesions, and the bacteria become active in them after the return of more favorable conditions.

The pure cultures isolated from the leaf lesions have been used for making inoculations into seedling apple trees.

1. Ibid, p. 7.

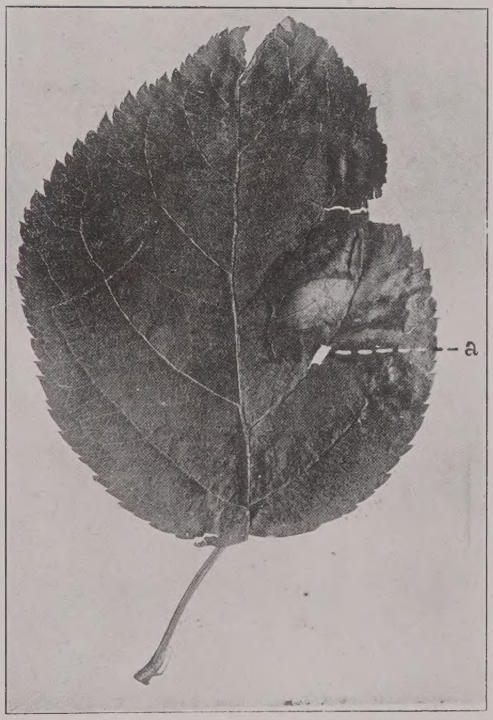

Fig. 3. Apple leaf with a well-developed lesion showing place (a) from which tissue was removed to make isolation of *Bacillus amylovorus* by the poured plate method.

The trees to be inoculated were placed in the inocluation chamber and kept well watered for 48 hours previous to introducing the bacteria into the tips just back of the terminal bud. The inoculations were made July 31st, and by August 8th the seedlings exhibited fire blight in severe and typical form. The microscopic examinations and the results from inoculations leave no doubt that the leaf lesions described above were due to *Bacillus amylovorus.*

To what extent twig blight is caused by the advance of the bacteria down the leaf petiole and thus into the twig has not been determined. This must be left undecided until the work of another season. It seems probable that we have here an explanation for many infections which have been attributed to insects. This information is also of some fundamental importance as it may explain why blight reappears in certain cases when all cankers and twig blight have been removed. It certainly complicates the practice of blight control by the cutting out method.